PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
99-79		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL00/00525	04/09/2000	05/09/1999
International Patent Classification (IPC) or national classification and IPC C07K14/00		
Applicant YEDA RESEARCH AND DEVELOPMENT CO. LTD. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09/03/2001	12.12.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Rojo Romeo, E Telephone No. +49 89 2399 7321

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IL00/00525

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-28 as originally filed

Claims, No.:

1-10 as originally filed

Drawings, sheets:

1/7-7/7 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

☐ copy of the earlier application whose priority has been claimed.

☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-4, 6-10 (partially).

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion

could be formed.

☒ no international search report has been established for the said claims Nos. 1-4, 6-10 (partially).

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	5 (entirely); 4, 6-10 (partially)
	No:	Claims	1-3 (partially)
Inventive step (IS)	Yes:	Claims	
	No:	Claims	5 (entirely); 1-4, 6-10 (partially)
Industrial applicability (IA)	Yes:	Claims	1-8, 10
	No:	Claims	9 (see separate sheet)

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item II

Priority

The right of priority can be acknowledged.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

As mentioned by the ISA, the present claims 1-4 and 6-10 relate to compounds defined by reference to a desirable characteristic or property, namely the inhibition of VEGF action or synthesis or the inhibition of angiogenesis, without giving any structural and essential characteristics of the compounds.

Consequently, the application was searched as far as it concerns the compounds mentioned on page 18 lines 3-9, page 18 lines 20-22 and claim 5.

As a result, the present claims are examined only as far as they concern said compounds.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents cited in the International Search Report:

D1: WO 97 27286 A (PROGENITOR INC) 31 July 1997 (1997-07-31)

D2: SIERRA-HONIGMANN M ROCIO ET AL: 'Biological action of leptin as an angiogenic factor.' SCIENCE (WASHINGTON D C), vol. 281, no. 5383, 11 September 1998 (1998-09-11), pages 1683-1686, XP002161601 ISSN: 0036-8075

D3: LAFONTAN MAX ET AL: 'Leptin and angiogenesis.' M-S (MEDECINE SCIENCES), vol. 15, no. 3, March 1999 (1999-03), pages 382-386, XP000982571 ISSN: 0767-0974

D4: WO 98 48831 A (RUBINSTEIN MENACHEM ;COHEN BATYA (IL); BARKAN DALIT (IL); YEDA RES) 5 November 1998 (1998-11-05)

Document D1 concerns the use of leptin for promoting angiogenesis, alone or in combination with cytokines such as VEGF. Moreover, this document suggests the use of leptin to suppress tumour growth by inducing terminal differentiation of certain tumour cells such as leukemic cells which express the leptin receptor.

D2 discloses the role of leptin as angiogenic factor, discussing the role of leptin-induced angiogenesis may assist in heat dissipation at sites of active thermogenesis in the body, including adipose tissue and may play a role in the modulation of adipose tissue mass.

D3 is a review addressing the role of leptin as angiogenic modulator, stressing its role in the development of the fat tissue but also in the neovascularisation of certain tumours, wound healing and oocyte and embryo maturation. This document also suggests the use of leptin in the control of angiogenesis for the regulation of adipogenesis.

D4 discloses the use of leptin, leptin fusion proteins, leptin muteins, leptin receptor agonists, active fragments or fractions of any one thereof, active analogs or derivatives of any thereof, as an inhibitor of cell proliferation, e.g. as an inhibitor of the proliferation of cancer cells (e.g. human breast carcinoma; see page 3)

The present application concerns the use of leptin in the inhibition of endothelial cell proliferation. The examples show that the injection of leptin in female mice lacking endogenous leptin leads to the regression of blood vessels in the adipose tissue, that leptin induces angiopoietin 2 in adipose tissue and that the use of leptin plus a VEGF inhibitor (CSC) leads to adipose mass reduction.

1. Novelty (Art. 33(2) PCT)

   The use of leptin for the preparation of medicaments was known from prior art. However, not for the inhibition of endothelial cell proliferation. Since leptin was shown in prior art to promote angiogenesis (D1-D3), it is unclear why leptin should have now an opposite effect as that shown in the past. If the leptin used by the Applicant has other technical features than the leptin (sequence, concentrations used/obtained...) and leptin derivatives used in the past and shown to have the opposite effect, then the Applicant should explain what these differences are.

   In addition, the Applicant's attention is drawn to the fact that no data is provided that the inhibition of endothelial cell proliferation is reversible. Thus, claim 1 is drawn as a "result to be achieved" without the indication of the technical features necessary for achieving this result.

   At the time being, novelty cannot be acknowledged for claim 1 and dependent claims 2, 3.

In summary, claims 1-3 are not novel, and thus, not inventive.

2. Inventive step (Art. 33(3) PCT)

The problem underlying the present application is the provision of a composition leading to angiogenesis inhibition. The solution provided by the present application is a composition comprising leptin and a VEGF inhibitor.

VEGF inhibitors were already known in the art as angiogenesis inhibitors. Thus, in the absence of comparative data showing that the combination of VEGF inhibitors with leptin has an improved antiangiogenic activity (especially in the light of the controversial data published), inventive step cannot be acknowledged for claims 4-10.

For the sake of completeness, the Applicant's attention is drawn to the fact that both VEGF inhibitors and leptin have been implicated in tumour inhibition (see D1/D4). Moreover, leptin was already known as an angiogenesis modulator in fat tissue (see D2/D3).

The Applicant's attention is drawn to the fact that the intention of use does not limit the scope of a claim which is directed to a composition. The claim must be interpreted as being directed to a composition per se regardless of its use. Claims 6, 7 and 8 are, as claim 10, directed to a composition comprising leptin and a VEGF inhibitor. No unified criteria exist in the PCT as far as first medical use is concerned. The EPO, for instance, will allow claims in a form such as:"substance or composition X", followed by the indication of use ("for use as a medicament"). Or in the case of known compounds (as it is the case here) drawn as a second medical use.

Consequently, claims 1-10 lack inventive step.

3. Industrial applicability (Art. 33(4) PCT)

For the assessment of the present claim 9 on the question whether it is industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the

manufacture of a medicament for a new medical treatment.

Re Item VI

Certain documents cited

D5: WO99/59614

international publication date:	25.11.99
international filing date:	20.05.99
priority data:	20.05.98

Re Item VII

Certain defects in the international application

Concerning the expression "spirit and scope of the invention" found at page 28, the Applicant's attention is drawn to the Guidelines III-4.3a PCT.

Re Item VIII

Certain observations on the international application

1. Clarity (Art. 6 PCT)

1.1 In the absence of technical features defining these compounds, "leptins homologues or derivatives" have no technical meaning for the skilled person. Concerning this, the Applicant's attention is drawn to the fact that the claims must be clear without the context of the description.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
EINAV, Henry
Attn. EINAV ,Henry
Inter-Lab Ltd.
Science-based Industrial Park
76110 Ness-Ziona
ISRAEL

Date of mailing *(day/month/year)*	13/03/2001
Applicant's or agent's file reference: 99-79	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No.: PCT/IL 00/ 00525	International filing date *(day/month/year)* 04/09/2000

Applicant

YEDA RESEARCH AND DEVELOPMENT CO. LTD. et al.

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Catherine Humbert

Form PCT/ISA/220 (July 1998)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 00/00525

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/22 A61K48/00 A61P3/00 A61P9/00 A61P15/00
A61P35/00 A61P43/00 //(A61K38/22,31:52),(A61K38/22,38:17),
(A61K38/22,31:185),(A61K38/22,31:195),(A61K38/22,38:19),

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS, EPO-Internal, WPI Data, PAJ, MEDLINE, CANCERLIT, EMBASE, CHEM ABS Data, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 27286 A (PROGENITOR INC) 31 July 1997 (1997-07-31) page 29, line 14 - line 30 page 31, line 1 -page 34, line 8 page 52, line 11 -page 54, line 2 claims 26-31 ---	1-10
A	SIERRA-HONIGMANN M ROCIO ET AL: "Biological action of leptin as an angiogenic factor." SCIENCE (WASHINGTON D C), vol. 281, no. 5383, 11 September 1998 (1998-09-11), pages 1683-1686, XP002161601 ISSN: 0036-8075 the whole document --- -/--	1-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 February 2001	13/03/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Stein, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 00/00525

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 (A61K38/22,38:48)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LAFONTAN MAX ET AL: "Leptin and angiogenesis." M-S (MEDECINE SCIENCES), vol. 15, no. 3, March 1999 (1999-03), pages 382-386, XP000982571 ISSN: 0767-0974 the whole document ---	1-10
A	WO 98 48831 A (RUBINSTEIN MENACHEM ;COHEN BATYA (IL); BARKAN DALIT (IL); YEDA RES) 5 November 1998 (1998-11-05) page 2, line 20 -page 9, line 3 claims 1-27; examples 5-9 --- -/--	1-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 February 2001	

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl. Fax: (+31-70) 340-3016	Stein, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 00/00525

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 59614 A (UNIV YALE ;SIERRA HONIGMANN ROCIO M (US)) 25 November 1999 (1999-11-25) the whole document, especially examples 2,3,5-13 and claims 1-9	1-10

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-4,6-10 partially

Present claims 1-4 and 6-10 relate to compounds defined by reference to a desirable characteristic or property, namely the inhibition of VEGF action or synthesis or the inhibition of angiogenesis. However these claims lack any structural and essential characteristics of the compounds.
The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compounds by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the compounds mentioned on page 18 lines 3-9, page 18 lines 20-22 and claim 5 of the application.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL 00/00525

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 9 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 1-4,6-10 partially
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IL 00/00525

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9727286 A	31-07-1997	AU 1831197 A	20-08-1997
		CA 2244693 A	31-07-1997
		EP 0892849 A	27-01-1999
WO 9848831 A	05-11-1998	AU 7076298 A	24-11-1998
		BG 103832 A	31-10-2000
		CN 1261802 T	02-08-2000
		EP 0981365 A	01-03-2000
		HU 0002427 A	28-12-2000
		NO 995267 A	28-12-1999
		PL 336582 A	03-07-2000
		SK 147199 A	12-06-2000
		ZA 9803608 A	02-11-1998
WO 9959614 A	25-11-1999	AU 4672199 A	06-12-1999

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
99-79		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/IL 00/00525	04/09/2000	05/09/1999
Applicant		
YEDA RESEARCH AND DEVELOPMENT CO. LTD. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[X] None of the figures.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-4,6-10 partially

Present claims 1-4 and 6-10 relate to compounds defined by reference to a desirable characteristic or property, namely the inhibition of VEGF action or synthesis or the inhibition of angiogenesis. However these claims lack any structural and essential characteristics of the compounds.
The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compounds by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the compounds mentioned on page 18 lines 3-9, page 18 lines 20-22 and claim 5 of the application.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

[redacted]ational Application No
PCT/IL 00/00525

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/22 A61K48/00 A61P3/00 A61P9/00 A61P15/00
A61P35/00 A61P43/00 //(A61K38/22,31:52),(A61K38/22,38:17),
(A61K38/22,31:185),(A61K38/22,31:195),(A61K38/22,38:19),

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS, EPO-Internal, WPI Data, PAJ, MEDLINE, CANCERLIT, EMBASE, CHEM ABS Data, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 27286 A (PROGENITOR INC) 31 July 1997 (1997-07-31) page 29, line 14 - line 30 page 31, line 1 -page 34, line 8 page 52, line 11 -page 54, line 2 claims 26-31 ---	1-10
A	SIERRA-HONIGMANN M ROCIO ET AL: "Biological action of leptin as an angiogenic factor." SCIENCE (WASHINGTON D C), vol. 281, no. 5383, 11 September 1998 (1998-09-11), pages 1683-1686, XP002161601 ISSN: 0036-8075 the whole document --- -/--	1-10

[X] Further documents are listed in the continuation of box C.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 February 2001	13/03/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Stein, A

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

[redacted]ational Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 (A61K38/22,38:48)

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Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LAFONTAN MAX ET AL: "Leptin and angiogenesis." M-S (MEDECINE SCIENCES), vol. 15, no. 3, March 1999 (1999-03), pages 382-386, XP000982571 ISSN: 0767-0974 the whole document ---	1-10
A	WO 98 48831 A (RUBINSTEIN MENACHEM ;COHEN BATYA (IL); BARKAN DALIT (IL); YEDA RES) 5 November 1998 (1998-11-05) page 2, line 20 -page 9, line 3 claims 1-27; examples 5-9 ---	1-10

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[X] Further documents are listed in the continuation of box C.

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"O" document referring to an oral disclosure, use, exhibition or other means

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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Date of the actual completion of the international search	Date of mailing of the international search report
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European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Stein, A

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INTERNATIONAL SEARCH REPORT

[redacted]ational Application No
PCT/IL 00/00525

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 59614 A (UNIV YALE ;SIERRA HONIGMANN ROCIO M (US)) 25 November 1999 (1999-11-25) the whole document, especially examples 2,3,5-13 and claims 1-9	1-10

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IL 00/00525

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9727286 A	31-07-1997	AU 1831197 A	20-08-1997
		CA 2244693 A	31-07-1997
		EP 0892849 A	27-01-1999
WO 9848831 A	05-11-1998	AU 7076298 A	24-11-1998
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		ZA 9803608 A	02-11-1998
WO 9959614 A	25-11-1999	AU 4672199 A	06-12-1999

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(54) Title: USE OF LEPTIN IN INHIBITION OF ENDOTHELIAL CELL PROLIFERATION

(57) Abstract: Disclosed is the use of leptin, optionally together with VEGF inhibitors, in inhibition of endothelial cell proliferation and modulation of angiogenesis.

USE OF LEPTIN IN INHIBITION OF ENDOTHELIAL CELL PROLIFERATION

Field of the Invention

The present invention relates to reversible inhibition of endothelial cell proliferation and to modulation of angiogenesis in the female reproductive system. More particularly, the present invention relates to the use of leptin or leptin homologues or derivatives, optionally together with inhibitors of VEGF action or inhibitors of VEGF synthesis, in the preparation of a medicament for inhibiting angiogenesis or modulating angiogenic processes. The present invention further relates to the use of leptin or leptin homologues or derivatives, together with inhibitors of VEGF action or inhibitors of VEGF synthesis, in the preparation of a medicament for modulation of angiogenesis in the female reproductive system.

Background of the Invention

As used herein, the term "angiogenesis" means the generation of new blood vessels into a tissue or organ. Under normal physiological conditions, humans or animals undergo angiogenesis only in very specific restricted situations. For example, angiogenesis is normally observed in wound healing, fetal and embryonic development, formation of the corpus luteum, endometrium and placenta and growth of adipose tissue.

The term "endothelium" means a thin layer of flat epithelial cells that lines serous cavities, lymph vessels, and blood vessels. Both controlled and

uncontrolled angiogenesis are thought to proceed in a similar manner. Endothelial cells and pericytes, surrounded by a basement membrane, form capillary blood vessels. Angiogenesis begins with the erosion of the basement membrane by enzymes released by endothelial cells and leukocytes. The endothelial cells, which line the lumen of blood vessels, then protrude through the basement membrane. Angiogenic stimulants induce the endothelial cells to migrate through the eroded basement membrane. The migrating cells form a "sprout" off the parent blood vessel, where the endothelial cells undergo mitosis and proliferate. The endothelial sprouts merge with each other to form capillary loops, creating the new blood vessel.

Persistent, unregulated angiogenesis occurs in a multiplicity of disease states, tumor metastasis and abnormal growth by endothelial cells and supports the pathological damage seen in these conditions. The diverse pathological disease states in which unregulated angiogenesis is present have been grouped together as angiogenic dependent or angiogenic associated diseases. The hypothesis that tumor growth is angiogenesis-dependent was first proposed in 1971. (Folkman J., Tumor angiogenesis: Therapeutic implications. N. Engl. J. Med. 285:1182-1186, 1971). In its simplest terms it states: "Once tumor 'take' has occurred, every increase in tumor cell population must be preceded by an increase in new capillaries converging on the tumor." Tumor 'take' is currently understood to indicate a prevascular phase of tumor growth in which a population of tumor cells occupying a few cubic millimeters volume and not exceeding a few million cells, can survive on existing host microvessels. Expansion of tumor volume beyond this phase requires the induction of new capillary blood vessels. For example, pulmonary micrometastases in the early prevascular phase in mice would be undetectable except by high power

microscopy on histological sections.

Vascular morphogenesis is regulated by the hypoxia-induced vascular endothelial growth factor (VEGF) and its endothelial cell receptors Flk1 and Flt1. Two other angiogenic factors, angiopoietin-1 and 2 (Ang1 and Ang2), which bind to a common endothelial cell receptor (Tie2), were identified (S. Davis, et al., *Cell* **87**, 1161-1169, 1996; P. C. Maisonpierre, et al., *Science* **277**, 55-60, 1997). Ang1 is a receptor agonist (C. Suri, et al., *Science* **282**, 468-471, 1998), constitutively expressed in many tissues, whereas Ang2 is a receptor antagonist, whose expression is limited to sites of vascular remodeling. So far, Ang2 was identified in fetal tissues, in endothelial cells, in smooth muscle cells and in female reproductive organs of adult mice and humans (P. C. Maisonpierre, et al., *Science* **277**, 55-60, 1997; B. Witzenbichler, P. C. Maisonpierre, P. Jones, G. D. Yancopoulos, J. M. Isner, *J Biol Chem* **273**, 18514-18521, 1998; S. J. Mandriota, M. S. Pepper, *Circ Res* **83**, 852-859, 1998). Both VEGF and Ang2 are up-regulated in female reproductive organs upon vascular morphogenesis, whereas only Ang2 is expressed upon blood vessel regression. Ang2 probably marks these vessels for regression by an apoptotic mechanism, although induction of apoptosis by Ang2 in cultured endothelial cells has not been obtained (B. Witzenbichler, P. C. Maisonpierre, P. Jones, G. D. Yancopoulos, J. M. Isner, *J Biol Chem* **273**, 18514-18521, 1998; J. Holash, et al., *Science* **284**, 1994-1998, 1999; D. Hanahan, *Science* **277**, 48-50, 1997).

A specific antibody against VEGF reduces microvessel density and causes "significant or dramatic" inhibition of growth of three human tumors, which rely on VEGF as their sole mediator of angiogenesis (in nude mice). The antibody does not inhibit growth of the tumor cells in vitro. (Kim K J, et al., Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumor

growth in vivo. Nature 362:841-844, 1993).

A specific angiogenesis inhibitor (AGM-1470) inhibits tumor growth and metastases in vivo, but is much less active in inhibiting tumor cell proliferation in vitro. It inhibits vascular endothelial cell proliferation half-maximally at 4 logs lower concentration than it inhibits tumor cell proliferation. (Ingber D, et al., Angioinhibins: Synthetic analogues of fumagillin which inhibit angiogenesis and suppress tumor growth. Nature, 48:555-557, 1990). There is also indirect clinical evidence that tumor growth is angiogenesis dependent.

Adipose tissue microcirculation is unique within the vascular system because of the capacity of this system to grow throughout most of adult life (D. L. Crandall, G. J. Hausman, J. G. Kral, *Microcirculation* **4**, 211-232, 1997). Indeed, brown and white adipose tissues have an extensive microvasculature and express high levels of VEGF (K. P. Claffey, W. O. Wilkison, B. M. Spiegelman, *J Biol Chem* **267**, 16317-16322, 1992; Q. X. Zhang, et al., *J Surg Res* **67**, 147-154, 1997). Thus, it is clear that angiogenesis plays a major role in the growth and maintenance of adipose tissue. If this angiogenic activity could be repressed or eliminated, then the adipose tissue will regress.

Obesity, defined as an excess of body fat relative to lean body mass, is associated with important psychological and medical morbidities, the latter including hypertension, elevated blood lipids, and Type II or non-insulin-dependent diabetes mellitus (NIDDM). There are 6-10 million individuals with NIDDM in the U.S., including 18% of the population of 65 years of age (Hanis et al., Ira. J. Obes., 11:275-283, 1987). Approximately 45 % of males and 70% of females with NIDDM are obese, and their diabetes is substantially improved or eliminated by weight reduction (Harris, Diabetes Care, 14(3):639-648, 1991).

Y. Zhang et al (Nature, 372, 425-431, 1994) suggest that one of the molecules which plays a key role in energy balance regulation is the ob protein also termed leptin. Zhang et al also report the cloning and sequencing of both mouse and human leptin. United Kingdom patent specification No. 2292382 relates inter alia to polypeptides, ob polypeptides or allelic variants or analogs thereof and their use for modulating body weight. In particular, GB 2292382 discloses that leptins and certain analogs thereof, such as agonists, would be useful for the treatment of obesity. Indeed, it was found that the adipocyte-derived leptin regulates food intake in rodents through its action on an hypothalamic receptor. Yet, later studies have shown that serum leptin is elevated in obese individuals and that there is a direct correlation between serum leptin and the body mass index (weight in kg divided by squared height in m.). The discrepancy between leptin's effect as an inhibitor of food intake and the high levels of leptin in obese individuals led to the theory of "leptin resistance", a term suggesting that obese individuals do not respond to their high leptin levels and maintain their high body mass. Thus it is clear that leptin by itself is not efficient in reducing the adipose tissue mass (P. Prolo, M. L. Wong, J. Licinio, *Int J Biochem Cell Biol* **30**, 1285-1290, 1998).

There exists therefore a need for a composition and method which can inhibit the unwanted growth of blood vessels, especially into tumors and adipose tissues. The composition should also be able to modulate the formation of capillaries in other angiogenic processes, such as wound healing and reproduction. The composition and method for inhibiting angiogenesis should preferably be non-toxic and produce few side effects. If angiogenic activity could be repressed or eliminated, then tumor, although present, would not grow and adipose tissue will regress. In the disease state, prevention of angiogenesis could

avert the damage caused by the invasion of the new microvascular system. Therapies directed at control of the angiogenic processes could lead to the abrogation or mitigation of these diseases.

Mice lacking leptin are infertile because leptin is required for release of gonadotropin-releasing hormone (GN-RH) from the hypothalamus. GN-RH acts on the pituitary gland and is essential for the release of the gonadotropins FSH and LH. Indeed, injection of leptin to leptin-defficient mice resuced their sterility. Feamales who have low adipose tissue mass, as the case of athletes or anorexia nervosa patients are infertile due to insufficient level of the adipose tissue-produced leptin.

One of the characteristics of the estrous cycle is ovarian angiogenesis, which takes place during the maturation of the follicle in the ovary. Rupture of the follicle and formation of the corpus luteum are associated with extensive blood vessel regression. These tissues were shown to express VEGF and Ang2. Therapies directed at control of angiogenic processes in the female reproductive system could regulate fertility.

Summary of the Invention

The present invention relates to the use of leptin, a leptin homologue or a derivative thereof, optionally together with an inhibitor of VEGF action or of VEGF synthesis, in the preparation of a medicament reversibly inhibiting endothelial cell proliferation.

In one aspect, the invention relates to the use of leptin, a leptin homologue or a derivative thereof in the preparation of a medicament for modulating angiogenic processes.

More particularly, the use in inhibition of angiogenisis is contemplated.

In another aspect, the invention provides for the use of leptin, a leptin homologue or a derivative thereof together with an inhibitor of VEGF action or VEGF synthesis in the preparation of a medicament for regulating fertility in a mammal.

Any known pharmaceutically acceptable VEGF inhibitor may be employed in accordance with the invention.

The invention also relates to pharmaceutical compositions modulating angiogenic processes or body weight or fertility comprising leptin, a leptin homologue or a leptin derivative optionally together with an inhibitor of VEGF action or VEGF synthesis.

Preferably the composition is employed in angiogenesis mediated diseases.

Brief Description of the Figures

Figure 1 shows a leptin induced blood vessel regression and apoptosis in adipose tissues of C57BL-*ob*$^{-/-}$ mice. C57CB-*ob*$^{-/-}$ mice were injected with murine leptin (2x1 μg/g) at time 0 and 9 h. Abdominal adipose tissues were removed at 24 and 48 h. Blood vessels were visualized in tissue sections by immunostaining with antibodies to Factor VIII (DAKO A/S, Denmark). Note that the number of stained blood vessels has decreased in 24 and 48 h post injection.

Figure 2 shows a dose-response curve of the blood vessel regression in adipose tissue of *ob*$^{-/-}$ mice 24 and 48 h post leptin injection.

Figure 3 shows a time course of the blood vessel regression in adipose tissues of *ob*$^{-/-}$ mice injected with murine leptin (2x1 μg/g).

Figure 4 shows a leptin-mediated induction of angiopoietin-2 (Ang2) as analyzed by reverse transcription-PCR of RNA from adipose tissues. Lane 1, control (no RNA); lane 2, RNA of adipose tissue from normal C57BL mouse; lane 3, RNA of adipose tissue from C57BL mouse injected with leptin (2x5 μg/g) lane 4, RNA of adipose tissue from C57BL-*ob*$^{-/-}$ mouse; lane 5, RNA of adipose tissue from C57BL-*ob*$^{-/-}$ mouse injected with leptin (2x5 μg/g). PCR reactions were terminated before saturation. PCR primers of Ang2, GeneBank Accession no. AF004326, corresponded to positions 637-657 (sense) and 1167-1147 (reverse).

Figure 5 shows a time course of Ang2 induction by leptin in adipose tissue of *ob*$^{-/-}$ mice. Leptin (2x5 μg/g) was administered to C57BL-*ob*$^{-/-}$ mice, total adipose RNA was extracted at the indicated times and analyzed by RNA blotting with probes to mouse Ang2, VEGF and actin.

Figure 6 shows a dose response of Ang2 induction in adipose tissue of *ob*$^{-/-}$ mice. Leptin was administered to C57BL-*ob*$^{-/-}$ mice and adipose proteins were extracted at 48 h. Ang2 was determined by immunoblot analysis (50 μg protein/lane) with specific antiserum (Santa Cruz Biotechnology, Santa Cruz, CA). The non-specific band (N.S.) serves for normalization of the immunoblot.

Figure 7 shows induction of Ang2 by leptin in cultured adipocytes. Cultures of undifferentiated mouse 3T3-F442A pre-adipocytes and differentiated adipocytes were induced with leptin (1 μg/ml). Total RNA was extracted at different time points and subjected to RNA blotting with probes to mouse Ang2, VEGF and actin. Notice the punctuate induction of Ang2 in adipocytes at 24 h and the reduction in VEGF level following differentiation of pre-adipocytes into mature adipocytes.

Detailed Description

Recently, leptin was reported to act as an angiogenic factor. It induced human umbilical vein endothelial cell proliferation in vitro, enhanced the formation of capillary-like tubes in vitro and induced neovascularization in corneas of mice and in a chic chorioallantoic membrane (M. R. Sierra-Honigmann, et al., *Science* **281**, 1683-1686, 1998; A. Bouloumie, H. C. Drexler, M. Lafontan, R. Busse, *Circ Res* **83**, 1059-1066, 1998). Although these studies suggested that leptin may induce angiogenesis at its site of production, the role of leptin as an angiogenic factor in the adipose tissue and in tumors has not yet been studied.

It has been found in accordance with the present invention that leptin, optionally together with other agents, acts as inducer of blood vessel regression in tissues and in tumors. Leptin potently induces the expression of the angiostatic factor angiopoietin-2 (Ang2) in various tissues, including adipose tissues and tumors. Ang2 is angiostatic in the absence of VEGF. Thus leptin is effective for modulating angiogenesis, and inhibiting unwanted angiogenesis, especially angiogenesis-related to tumor growth, adipose tissue growth and the estrous cycle.

The present invention includes the use of leptin, or homologues of leptin, or derivatives of leptin, optionally together with one or more inhibitors of VEGF production or VEGF action (hereinafter: "VEGF inhibitors") as inhibitors of tumor angiogenesis and modulators of angiogenesis in the female reproductive organs.

The present invention also includes the use of leptin, or homologues of leptin, or derivatives of leptin, together with VEGF inhibitors to induce adipose-tissue regression and to modulate angiogenesis in the female

reproductive organs.

Administration of leptin, or homologues of leptin, or leptin derivatives, either alone or together with VEGF inhibitors to a human or animal with prevascularized metastasized tumors will prevent the growth or expansion of those tumors.

Administration of leptin, or homologues of leptin, or leptin derivatives, in combination with VEGF inhibitors or other inhibitors of angiogenesis to females will modulate angiogenesis in their reproductive organs.

Diseases and processes that are mediated by angiogenesis include, but are not limited to, hemangioma, solid tumors, blood borne tumors, leukemia, metastasis, telangiectasia, psoriasis, scleroderma, pyogenic granuloma, myocardial angiogenesis, Crohn's disease, plaque neovascularization, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis, corneal diseases, rubeosis, neovascular glaucoma, diabetic retinopathy, retrolental fibroplasia, arthritis, diabetic neovascularization, macular degeneration, wound healing, peptic ulcer, Helicobacter related diseases, fractures, keloids, vasculogenesis, hematopoiesis, ovulation, menstruation, placentation, and cat scratch fever.

Administration of leptin, or homologues of leptin, or leptin derivatives, together with VEGF inhibitors or other inhibitors of angiogenesis to human or animal will reduce abberant angiogenesis associated with the aforesaid diseases more effectively than the VEGF inhibitor alone or other inhibitors of angiogenesis when applied without leptin.

It is also possible to modulate angiogenic processes by gene therapy as will be described hereinafter.

The present invention includes the method of treating an

angiogenesis-mediated disease with an effective amount of leptin, or homologues of leptin, or a leptin derivatives, optionally together with VEGF inhibitors or other inhibitors of angiogenesis. The effective amount of leptin, or homologues of leptin, or a leptin derivatives, optionally together with VEGF inhibitors or other inhibitors of angiogenesis is administered to patients in a pharmaceutically acceptable composition.

It is to be understood that the present invention is contemplated to include the use of any homologues of leptin that induce endothelial inhibitory activity. Homologues of leptin refer to proteins, in which one or more of the amino acid residues of a natural leptin are replaced by different amino acid residues, or are deleted, or one or more amino acid residues are added to the natural sequence of leptin, without changing considerably the activity of the resulting products as compared with the wild type leptin. These homologues are prepared by known synthesis and/or by site-directed mutagenesis techniques, or any other known technique suitable therefor.

Any such homologue preferably has a sequence of amino acids sufficiently duplicative of that of leptin, such as to have substantially similar activity to leptin. One such activity is the ability of a leptin homologue to reduce the body weight of ob/ob mice. Thus, it can be determined whether any given homologue has substantially the same activity as leptin by means of routine experimentation.

In a preferred embodiment, any such mutein has at least 40% sequence identity or homology with the sequence of either leptin. More preferably, it has at least 50%, at least 60%, at least 70%, at least 80% or, most preferably, at least 90% sequence identity or homology thereto.

Homologues of leptin polypeptides, which can be used in accordance with

the present invention, or nucleic acid coding therefor, include a finite set of substantially corresponding sequences as substitution peptides or polynucleotides which can be routinely obtained by one of ordinary skill in the art, without undue experimentation, based on the teachings and guidance presented herein. For a detailed description of protein chemistry and structure, see Schulz, G.E. et al., *Principles of Protein Structure*, Springer-Verlag, New York, 1978; and Creighton, T.E., *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, 1983, which are hereby incorporated by reference. For a presentation of nucleotide sequence substitutions, such as codon preferences, see Ausubel et al, *supra*, at §§ A.1.1-A.1.24, and Sambrook et al, *supra*, at Appendices C and D.

Preferred changes for homologues in accordance with the present invention are what are known as "conservative" substitutions. Conservative amino acid substitutions of leptin polypeptides may include synonymous amino acids within a group which have sufficiently similar physicochemical properties that substitution between members of the group will preserve the biological function of the molecule, Grantham, Science, Vol. 185, pp. 862-864 (1974). It is clear that insertions and deletions of amino acids may also be made in the above-defined sequences without altering their function, particularly if the insertions or deletions only involve a few amino acids, *e.g.*, under thirty, and preferably under ten, and do not remove or displace amino acids which are critical to a functional conformation, *e.g.*, cysteine residues, Anfinsen, "Principles That Govern The Folding of Protein Chains", Science, Vol. 181, pp. 223-230 (1973). Proteins and muteins produced by such deletions and/or insertions come within the purview of the present invention.

Preferably, the synonymous amino acid groups are those defined in Table

I. More preferably, the synonymous amino acid groups are those defined in Table II; and most preferably the synonymous amino acid groups are those defined in Table III.

TABLE I

Preferred Groups of Synonymous Amino Acids

Amino Acid	Synonymous Group
Ser	Ser, Thr, Gly, Asn
Arg	Arg, Gln, Lys, Glu, His
Leu	Ile, Phe, Tyr, Met, Val, Leu
Pro	Gly, Ala, Thr, Pro
Thr	Pro, Ser, Ala, Gly, His, Gln, Thr
Ala	Gly, Thr, Pro, Ala
Val	Met, Tyr, Phe, Ile, Leu, Val
Gly	Ala, Thr, Pro, Ser, Gly
Ile	Met, Tyr, Phe, Val, Leu, Ile
Phe	Trp, Met, Tyr, Ile, Val, Leu, Phe
Tyr	Trp, Met, Phe, Ile, Val, Leu, Tyr
Cys	Ser, Thr, Cys
His	Glu, Lys, Gln, Thr, Arg, His
Gln	Glu, Lys, Asn, His, Thr, Arg, Gln
Asn	Gln, Asp, Ser, Asn
Lys	Glu, Gln, His, Arg, Lys
Asp	Glu, Asn, Asp
Glu	Asp, Lys, Asn, Gln, His, Arg, Glu
Met	Phe, Ile, Val, Leu, Met
Trp	Trp

TABLE II

More Preferred Groups of Synonymous Amino Acids

Amino Acid	Synonymous Group
Ser	Ser
Arg	His, Lys, Arg
Leu	Leu, Ile, Phe, Met
Pro	Ala, Pro
Thr	Thr
Ala	Pro, Ala
Val	Val, Met, Ile
Gly	Gly
Ile	Ile, Met, Phe, Val, Leu
Phe	Met, Tyr, Ile, Leu, Phe
Tyr	Phe, Tyr
Cys	Cys, Ser
His	His, Gln, Arg
Gln	Glu, Gln, His
Asn	Asp, Asn
Lys	Lys, Arg
Asp	Asp, Asn
Glu	Glu, Gln
Met	Met, Phe, Ile, Val, Leu
Trp	Trp

TABLE III

Most Preferred Groups of Synonymous Amino Acids

Amino Acid	Synonymous Group
Ser	Ser
Arg	Arg
Leu	Leu, Ile, Met
Pro	Pro
Thr	Thr
Ala	Ala
Val	Val
Gly	Gly
Ile	Ile, Met, Leu
Phe	Phe
Tyr	Tyr
Cys	Cys, Ser
His	His
Gln	Gln
Asn	Asn
Lys	Lys
Asp	Asp
Glu	Glu
Met	Met, Ile, Leu
Trp	Met

Examples of production of amino acid substitutions in proteins which can be used for obtaining homologues of leptin polypeptides or proteins for use in the

present invention include any known method steps, such as presented in US patents RE 33,653, 4,959,314, 4,588,585 and 4,737,462, to Mark et al; 5,116,943 to Koths et al., 4,965,195 to Namen et al; 4,879,111 to Chong et al; and 5,017,691 to Lee et al; and lysine substituted proteins presented in US patent No. 4,904,584 (Shaw et al).

In another preferred embodiment of the present invention, any homologue of leptin has an amino acid sequence essentially corresponding to that of leptin. The term "essentially corresponding to" is intended to comprehend proteins with minor changes to the sequence of the natural protein which do not affect the basic characteristics of the natural proteins, particularly insofar as their ability to induce angiostatic activity. The type of changes which are generally considered to fall within the "essentially corresponding to" language are those which would result from conventional mutagenesis techniques of the DNA encoding these proteins, resulting in a few minor modifications, and screening for the desired activity in the manner discussed above.

It is to be understood that the present invention is contemplated to include the use of any derivatives of leptin that induce endothelial inhibitory activity when applied optionally together with a VEGF inhibitor or other inhibitors of angiogenesis. The present invention includes the use of an entire leptin protein, the use of derivatives of the leptin protein and the use of biologically active fragments of the leptin protein. Derivatives of leptin according to the invention have one or more chemical moieties attached thereto, including water-soluble polymers such as polyethylene glycol. Polyethylene glycol derivatized derivatives can be mono-, di-, tri- or tetrapegylated e. g., N-terminal monopegylated. Preferred N-terminal monopeglyated derivatives of leptin, optionally having a (pegylated) methionine at the N-terminus.

Various inhibitors of VEGF activity or VEGF production have been described and may be used in combination with leptin in order to inhibit angiogenesis. Among these inhibitors are 3,7-dimethyl-1-propargylxanthine(DMPX), an A2-antagonist, 7-(beta-hydroxyethyl)theophylline, 8-phenyltheophylline, the adenosine A2 receptor antagonist CSC (8-(3-chlorostyryl)caffeine), theobromine, an antagonistic VEGF variant, the soluble VEGF receptor sFLT-1, Tranilast, 8-(3-oxo-4,5,6-trihydroxy-3h-xanthen-9-yl)-1-naphthoic acid, suramin and platelet factor-4 (E. Hashimoto, et al., *Biochem Biophys Res Commun* **204**, 318-24, 1994; S. Fischer, R. Knoll, D. Renz, G. F. Karliczek, W. Schaper, *Endothelium* **5**, 155-165, 1997; H. Takagi, G. L. King, G. S. Robinson, N. Ferrara, L. P. Aiello, *Invest Ophthalmol Vis Sci* **37**, 2165-2176, 1996; E. Barcz, et al., *Oncol Rep* **5**, 517-520, 1998; G. Siemeister, et al., *Proc Natl Acad Sci U S A* **95**, 4625-4629, 1998; W. Roeckl, et al., *Exp Cell Res* **241**, 161-170, 1998, S. Komaya et al., *Br J Pharmacol* **127**, 537-545, 1999: K. Igarashi et al., *Int J Mol Med* **2**, 211-215, 1998; J. Waltenberger et al., *J Mol Cell Cardiol* **28**, 1523-1529, 1996; S. Grengrinovitch et al., *J Biol Chem* **270**, 15059-15065, 1995).

Various inhibitors of angiogenesis have been described and may be used in combination with leptin in order to inhibit angiogenesis more effectively than when used alone. Among these inhibitors are K1-5 (Cao, R. et al, Proc Natl Acad Sci U S A, 96, 5728-5733, 1999), angiostatin, endostatin, BB-94 and AGM-1470 (Bergers G. et al, Science284, 808-812, 1999).

Also comprised by the present invention is the use of expression vectors encoding leptin or leptin homologues, provided by gene therapy, optionally together with inhibitors of VEGF action or production or other inhibitors of angiogenesis for inhibition of angiogenesis in tumors. Such medicaments can be

employed in therapeutic methods involving intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, nasal, oral or pulmonary delivery systems.

Also comprised by the present invention is the use of expression vectors encoding leptin or leptin homologues, provided by gene therapy, in combination with inhibitors of VEGF action or production or other inhibitors of angiogenesis for regression of adipose tissues. Such therapy may be useful in treating a disorder selected from the group consisting of diabetes, high blood pressure and high cholesterol and as part of combinative therapy with a medicament for treating such disorders. Such medicaments can be employed in therapeutic methods involving intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, nasal, oral or pulmonary delivery systems.

The angiogenesis mediated diseases include, but are not limited to, obesity; solid tumors; blood born tumors such as leukemias; tumor metastasis; benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; rheumatoid arthritis; psoriasis; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis; Osler-Webber Syndrome; myocardial angiogenesis; plaque neovascularization; telangiectasia; hemophiliac joints, angiofibroma; and wound granulation.

Leptin, or homologues of leptin, or leptin derivatives, optionally together with VEGF or other inhibitors of angiogenesis inhibitors are useful in the treatment of disease of excessive or abnormal stimulation of endothelial cells. These diseases include, but are not limited to, intestinal adhesions, Crohn's disease, arteriosclerosis, scleroderma, and hypertrophic scars, i.e., keloids.

Leptin, or homologues of leptin, or leptin derivatives, optionally together with VEGF inhibitors or other inhibitors of angiogenesis may be used in combination with other compositions and procedures for the treatment of diseases. For example, a tumor may be treated conventionally with surgery, radiation or chemotherapy combined with leptin, or homologues of leptin, or leptin derivatives, optionally together with VEGF inhibitors or other inhibitors of angiogenesis and then leptin, or homologues of leptin, or leptin derivatives, optionally together with VEGF inhibitors or other inhibitors of angiogenesis may be subsequently administered to the patient to extend the dormancy of micrometastases and to stabilize and inhibit the growth of any residual primary tumor.

Additionally, Leptin, or homologues of leptin, or leptin derivatives, optionally together with VEGF inhibitors or other inhibitors of angiogenesis, are combined with pharmaceutically acceptable excipients. Compositions suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the composition isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The compositions may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Compositions may optionally include sustained-release matrix, such as biodegradable polymers, to form therapeutic compositions. A sustained-release

matrix, as used herein, is a matrix made of materials, usually polymers, which are degradable by enzymatic or acid/base hydrolysis or by dissolution. Once inserted into the body, the matrix is acted upon by enzymes and body fluids. The sustained-release matrix desirably is chosen from biocompatible materials such as liposomes, polylactides (polylactic acid), polyglycolide (polymer of glycolic acid), polylactide co-glycolide (co-polymers of lactic acid and glycolic acid) polyanhydrides, poly(ortho)esters, polypeptides, hyaluronic acid, collagen, chondroitin sulfate, carboxylic acids, fatty acids, phospholipids, polysaccharides, nucleic acids, polyamino acids, amino acids such as phenylalanine, tyrosine, isoleucine, polynucleotides and polyvinylpyrrolidone.

A preferred biodegradable matrix is a matrix of one of either polylactide, polyglycolide, or polylactide co-glycolide (co-polymers of lactic acid and glycolic acid). The polymers being implanted in the vicinity of where drug delivery is desired, for example, at the adipose tissue or at a site of a tumor or implanted, so that the leptin, or leptin derivatives, optionally together with VEGF inhibitors or other inhibitors of angiogenesis is slowly released systemically. The biodegradable polymers and their use are described, for example, in detail in Brem et al., J. Neurosurg. 74:441-446 (1991), which is hereby incorporated by reference in its entirety.

The angiogenesis-modulating pharmaceutical compositions according to the present invention may be a solid, liquid or aerosol and may be administered by any known route of administration. Examples of solid therapeutic compositions include pills, creams, and implantable dosage units. The pills may be administered orally; the therapeutic creams may be administered topically. The implantable dosage units may be administered locally, for example at a tumor site, or may be implanted for systemic release of the therapeutic

angiogenesis-modulating composition, for example subcutaneously. Examples of liquid compositions include compositions adapted for injection subcutaneously, intravenously, intraarterially, and compositions for topical and intraocular administration. Examples of aerosol compositions include inhaler composition for administration to the lungs.

It should be understood that in addition to the ingredients, specifically mentioned above, the compositions according to the present invention may include other agents conventional in the art having regard to the type of composition in question. Optionally, cytotoxic agents may be incorporated or otherwise combined with leptin, or homologues of leptin, or leptin derivatives, optionally together with VEGF inhibitors or other inhibitors of angiogenesis, to provide dual therapy to the patient.

The compositions according to the invention can be administered by standard routes. In general, the combinations may be administered by the topical (including buccal and sublingual), or parenteral (including subcutaneous, intraperitoneal, intramuscular, intravenous, intradermal, intracerebral, intracerebroventricular, intracranial, intraspinal, intratracheal, and epidural), transdermal, intravaginal, intrauterine, oral, rectal, ophthalmic (including intravitreal or intracameral), or intranasal, administration.

Osmotic minipumps may also be used to provide controlled delivery of high concentrations of leptin, or leptin derivatives, optionally together with VEGF inhibitors or other inhibitors of angiogenesis through cannulae to the site of interest, such as directly into a metastatic growth or into the vascular supply to that tumor.

The dosage of the leptin, or leptin derivatives, optionally together with VEGF inhibitors or other inhibitors of angiogenesis of the present invention will

depend on the disease state or condition being treated and other clinical factors such as weight and condition of the human or animal and the route of administration of the compound. For treating humans or animals, between approximately 0.5 mg/kilogram to 10 mg/kilogram of the leptin or leptin homologue or leptin derivative can be administered, optionally together with a suitable dose of a VEGF inhibitor or inhibitor of VEGF production or other inhibitors of angiogenesis. Depending upon the half-life of the leptin or leptin homologue or leptin derivative in the particular animal or human, the leptin or leptin homologue or leptin derivative can be administered between several times per day to once a week. Preferred unit dosage compositions are those containing a daily dose or unit, daily sub-dose, or an appropriate fraction thereof, of the administered ingredient. The methods of the present invention contemplate single as well as multiple administrations, given either simultaneously or over an extended period of time.

The leptin or leptin homologue or leptin derivative compositions may conveniently be presented in unit dosage form and may be prepared by conventional pharmaceutical techniques. Such techniques include the step of bringing into association the active ingredient and the pharmaceutical carrier(s) or excipient(s). In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Preferred unit dosage compositions are those containing a daily dose or unit, daily sub-dose, or an appropriate fraction thereof, of the administered ingredient. It should be understood that in addition to the ingredients, specifically mentioned above, the compositions of the present invention may include other agents conventional in the art having regard to the type of composition in

question.

It is to be understood that the present invention has application for both human and veterinary use.

This invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

Examples

Example 1

Induction of blood vessel regression by leptin

To test leptin's effect on blood vessel homeostasis in adult adipose tissue, murine leptin (0.1-5 μg/g) was injected ip at time 0 and 9 h to 8-10 weeks old obese (C57BL-ob$^{-/-}$) female mice, lacking endogenous leptin. A noticeable weight loss was observed at 48 h in mice receiving ≥ 2x1 μg/g leptin (65.4±0.5 g vs. 62.7±1.0 g, n=6). Abdominal fat was removed and fixed 24 and 48 h after the first injection, and blood vessels were counted after staining paraffin sections with antibodies to Factor VIII (D.D. Wagner et al. J Cell Biol 95, 355-360 (1982)). A significant reduction in the number of blood vessels was observed (198±1 vessels per 5 high power fields (HPFs, x400) in control mice; 159±2.5 vessels per 5 HPFs in leptin-treated mice (2x1 μg/g) at 24 h and 106±7.5 vessels per 5 HPFs at 48 h. **Figure 1** shows micrographs of blood vessels in adipose

tissue sections. **Figure 2** shows the dose-response curve of the blood vessel regression and **Figure 3** shows the time course of this regression.

Example 2

Leptin induces Angiopoietin 2 (Ang2) in adipose tissues

The mechanism by which leptin induced the blood vessel regression in adipose tissues was studied by measuring its effect on the expression level of angiogenic and angiostatic factors. Total RNA was isolated from adipose tissue of C57BL and C57BL-*ob*$^{-/-}$ mice at time 0 and 24 h after the first leptin administration. Total RNA was isolated with the TRI reagent. Reverse transcription was carried out in 20 µl volume using RNase H$^-$ reverse transcriptase (SuperScript II, GIBCO-BRL) with 1 µg $(N)_6$ random primer (New England Biolabs) according to the manufacturer's instructions. Aliquot (2 µl) of the reverse transcription product was used for PCR with VENT DNA polymerase (New England Biolabs) and the following sense and antisense primers: muAng-2 mRNA, GeneBank Accession No. AF4326 nucleotides 637-657 and 1147-1167; muVEGF, GeneBank Accession No. M95200 nucleotides 385-406 and 962-980; muActin mRNA, GeneBank Accession No. J00691 nucleotides 1670-1691 and 2452-2431. PCR reactions were terminated before saturation. It was found that Ang2 mRNA is expressed in adipose tissue of normal mice and not in that of the *ob*$^{-/-}$ mice. Furthermore, injection of leptin induced the expression of Ang2 in both types of mice **(Figure 4)**. These results demonstrate that leptin is a potent inducer of the angiostatic factor Ang2.

The levels and induction of Ang2 mRNA by leptin in the adipose tissue of

ob$^{-/-}$ mice was then studied by RNA blotting with specific probes to Ang2 and VEGF. Total RNA from adipose tissue was isolated with the TRI reagent kit (Molecular Research Center Inc.). Samples of RNA (15 μg) were resolved by electrophoresis through 1% agarose gel in MOPS-formaldehyde buffer, transferred to nylon membrane (Hybond N, Amersham) in 20XSSC buffer and the membrane was then heated for 2 hours at 80°C in a vacuum oven. The membrane was pre-hybridized (6 h, 42°C) with denatured Salmon-sperm DNA (100 μg/ml in 50% formamide, 5xSSC, 4xDenhard's solution and 0.5% SDS). A [^{32}P]dCTP DNA probe (1x10^{6} cpm/ml), prepared by random priming, was then added and hybridization continued for 18 hours at 42°C. The membrane was then washed at room temperature (1xSSC, 0.1% SDS twice, 0.25xSSC, 0.1% SDS and 0.1xSSC, 0.1% SDS twice, 30 min. each wash) and autoradiographed. Blots were then re-hybridized with ^{32}P-labeled probe corresponding to mouse actin to show equal amounts of RNA in the blot. A significant induction of Ang2 was obtained following leptin administration (2.9±0.4 fold, $P<0.05$, n=3 at 24 h and 16.0±0.31 fold, $P<0.01$, n=3 at 48 h; **Figure 5**). The kinetics of Ang2 expression corresponded to that of the apoptosis and blood vessel regression. The level of VEGF mRNA was only slightly induced (1.4±0.1 fold, n=3 at 48 h; **Figure 5**).

The dose-response of leptin-induced Ang2 in adipose tissues was studied by immunoblotting 48 h after the first injection of leptin. Cell extracts from adipose tissue were isolated using the TRI reagent kit (Molecular Research Center Inc.) in parallel to total RNA extraction. Fifty micrograms protein were separated on 10% SDS-polyacrylamide gel. Immunoblot analysis was carried out with 5 μg of a specific goat anti-human Ang-2 antibody. Ang2 was below the level of detection in adipose tissue of control *ob*$^{-/-}$ mice, whereas administration

of 2x1 μg/g leptin was sufficient for high level induction of Ang2 (**Figure 6**).

Example 3

Leptin induces Angiopoietin 2 (Ang2) in cultured adipocytes

Several peripheral activities of leptin were previously reported (M. R. Sierra-Honigmann, et al., *Science* **281**, 1683-1686, 1998; A. Bouloumie, H. C. Drexler, M. Lafontan, R. Busse, *Circ Res* **83**, 1059-1066,1998; B. Cohen, D. Novick, M. Rubinstein, *Science* **274**, 1185-1188, 1996; D. Barkan, et al., *Endocrinology* **140**, 1731-1738, 1999). To test if leptin may act directly on adipocytes, we studied the effect of leptin on murine 3T3-F442A pre-adipocytes, known to give rise to adipose-like tissue upon implantation in athymic mice (H. Green, O. Kehinde, *J Cell Physiol* **101**, 169-171, 1979); S. Mandrup, T. M. Loftus, O. A. MacDougald, F. P. Kuhajda, M. D. Lane, *Proc Natl Acad Sci U S A* **94**, 4300-4305,1997). Swiss 3T3 F442A murine pre-adiopcytes (H. Green, O. Kehinde, *Cell* **5**, 19-27, 1975) were grown in DMEM (GIBCO) and 10% calf serum. For differentiation, confluent cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) for six days. Medium was replaced every 48 hours. By the end of the period most of the cells acquired the characteristic adipocyte morphology as determined by biochemical and morphological criteria. Leptin (1 μg/ml) was added to cultures of differentiated and non-differentiated cells. RNA was isolated from the cultured cells as described for the adipose tissues of Example 2 and subjected to RNA blot analysis. It was found that leptin induced Ang2 mRNA expression in differentiated 3T3-F442A adipocytes and not in pre-adipocytes. Ang2 mRNA appeared punctuate at 24 h. VEGF mRNA was constitutively expressed in

pre-adipocytes and was further induced by leptin. The level of VEGF mRNA was significantly lower in mature adipocytes and was not significantly induced by leptin **(Figure 7)**. These result suggest that leptin induces an angiostatic signal in mature adipocytes and angiogenic signals in pre-adipocytes.

Example 4

Effect of leptin plus a VEGF inhibitor on adipose mass reduction

The angiostatic activity of leptin-induced Ang2 is reversed in the presence of VEGF. Furthermore, a modest induction of VEGF by leptin was noticed in the previous examples. Therefore, murine leptin (0.1-5 μg/g) is injected ip at time 0 and 9 h to 8-10 weeks old obese (C57BL-ob$^{-/-}$) female mice, lacking endogenous leptin. In parallel, 8-10 weeks old obese (C57BL-ob$^{-/-}$) female mice were injected at times 0 and 9 h ip with murine leptin (0.1-5 μg/g) together with the adenosine 2 receptor antagonist CSC, known to function as a VEGF inhibitor (H. Takagi, G. L. King, G. S. Robinson, N. Ferrara, L. P. Aiello, *Invest Ophthalmol Vis Sci* **37**, 2165-2176, 1996). A noticeable weight loss was observed at 48 h in mice receiving ≥ 2x1 μg/g leptin alone (65.4±0.5 g vs. 62.7±1.0 g, n=6). A significantly higher weight loss is observed in mice treated with a combination of leptin and CSC.

It should be understood that the foregoing relates only to preferred embodiments of the present invention, and that numerous modifications or alterations may be made therein without departing from the spirit and the scope of the invention as set forth in the appended claims.

CLAIMS:

1. The use of leptin or a leptin homologue or derivative optionally together with an inhibitor of VEGF action or of VEGF synthesis and/or an inhibitor of angiogenesis, in the preparation of a medicament reversibly inhibiting endothelial cell proliferation.

2. The use according to claim 1 for modulating angiogenic processes.

3. The use according to claim 2 for inhibiting angiogenesis.

4. The use according to claim 3, including an angiogenesis inhibitor.

5. The use according to anyone of the preceding claims wherein the VEGF inhibitor is selected from DMPX, an A2-antagonist 7-(betahydroxyethyl)theophylline, 8-phenyltheophylline, the adenosine A2 receptor antagonist CSC, theobromine, an antagonistic VEGF variant, sFLT-1, Tranilast, 8-(3-oxo-4,5,6-trihydroxy-3h-xanthen-9-yl)-1-naphthoic acid, suramin and platelet factor-4 .

6. A pharmaceutical composition for reversibly inhibiting endothelial cell proliferation comprising leptin or a leptin homologue or derivative optionally together with an inhibitor of VEGF action or of VEGF synthesis and/or an inhibitor of angiogenesis.

7. A pharmaceutical composition according to claim 6 for modulating

angiogenic processes.

8. A pharmaceutical composition according to claim 7 for inhibiting angiogenesis.

9. A method for reversibly inhibiting endothelial cell proliferation in mammals comprising administering to a subject a pharmaceutical composition according to any one of claims 6 to 9 in a suitable dosage form and by a suitable route of administration.

10. A mixture comprising leptin and a VEGF inhibitor.

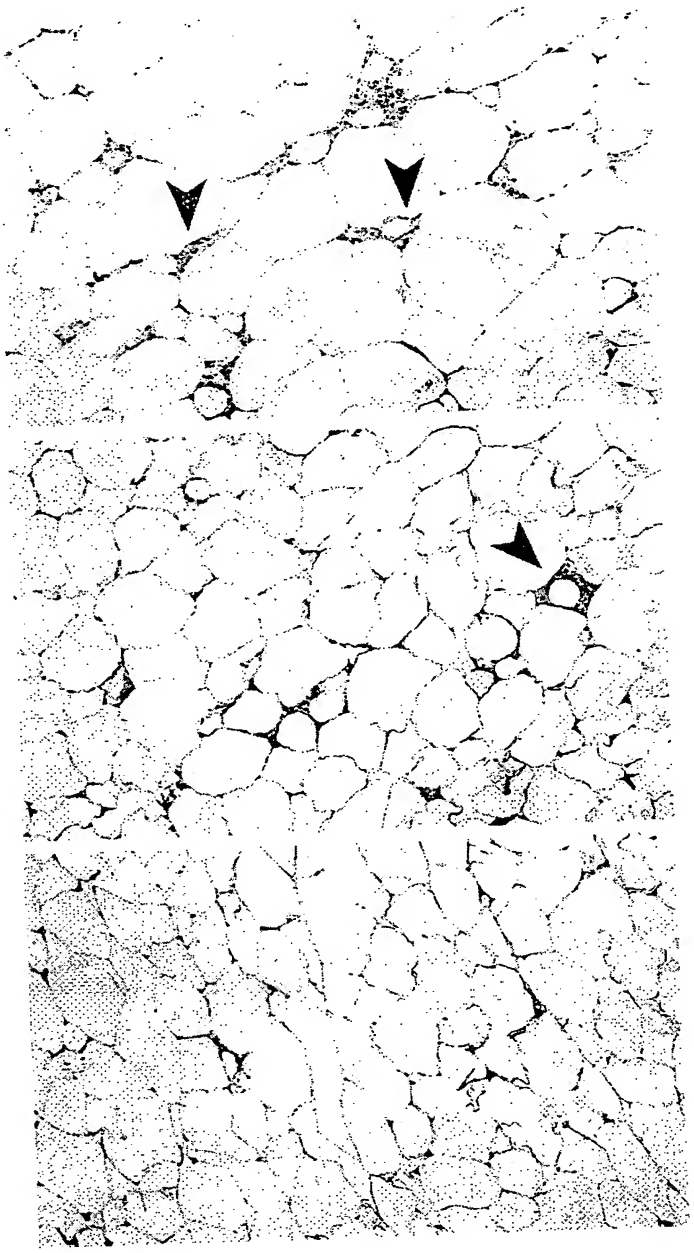

FIGURE 1

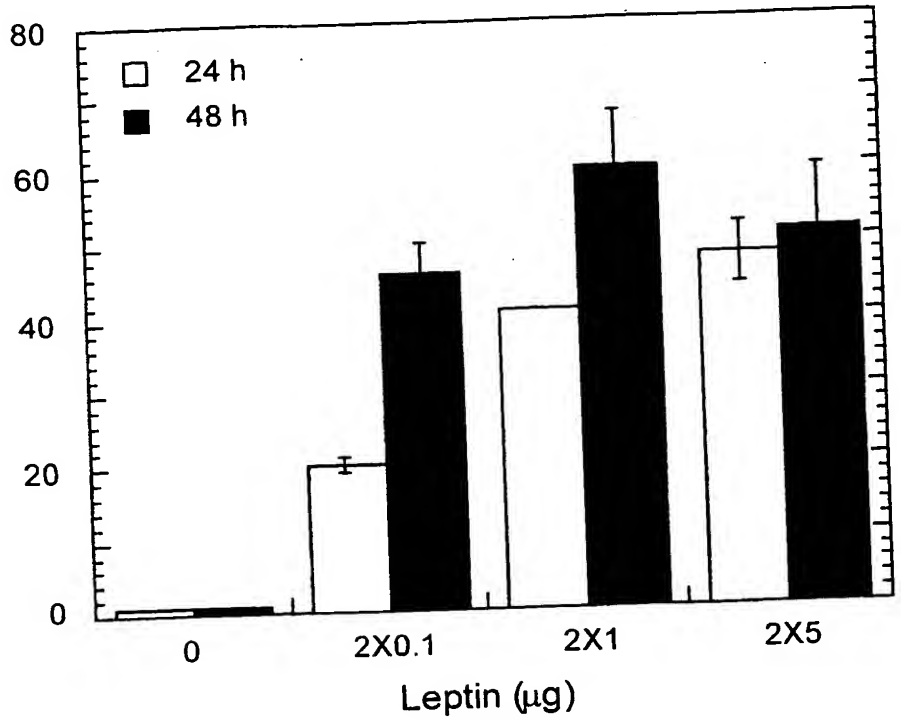

FIGURE 2

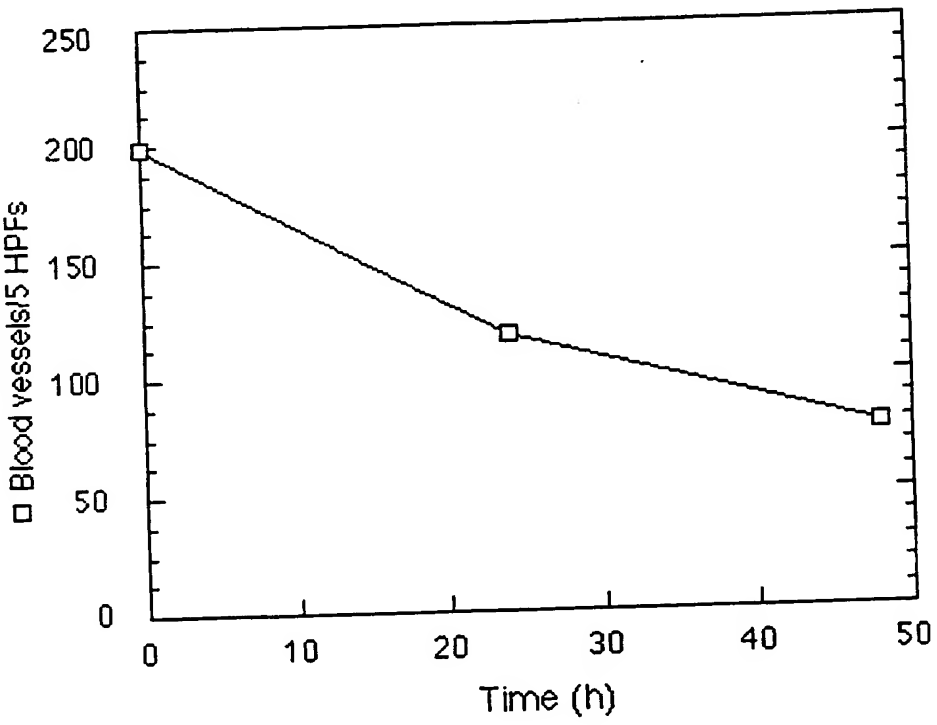

FIGURE 3

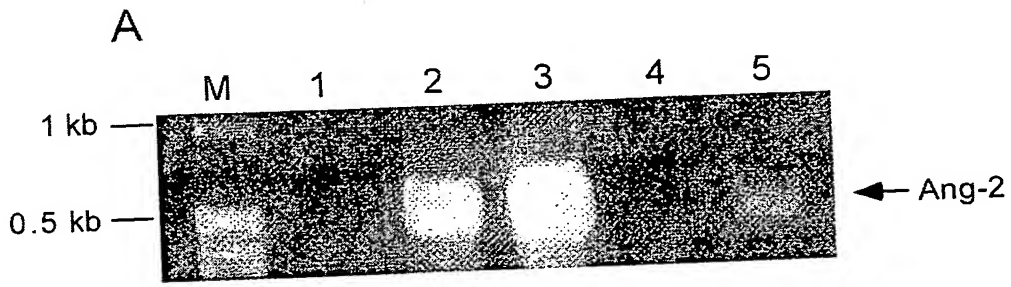

FIGURE 4

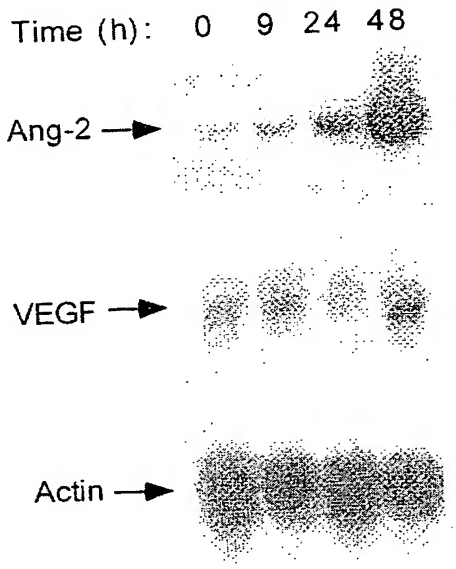

FIGURE 5

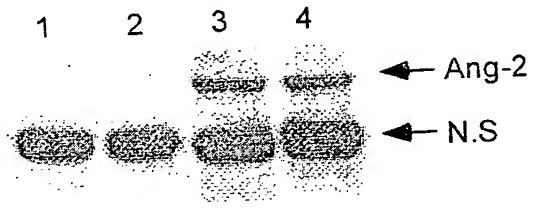

FIGURE 6

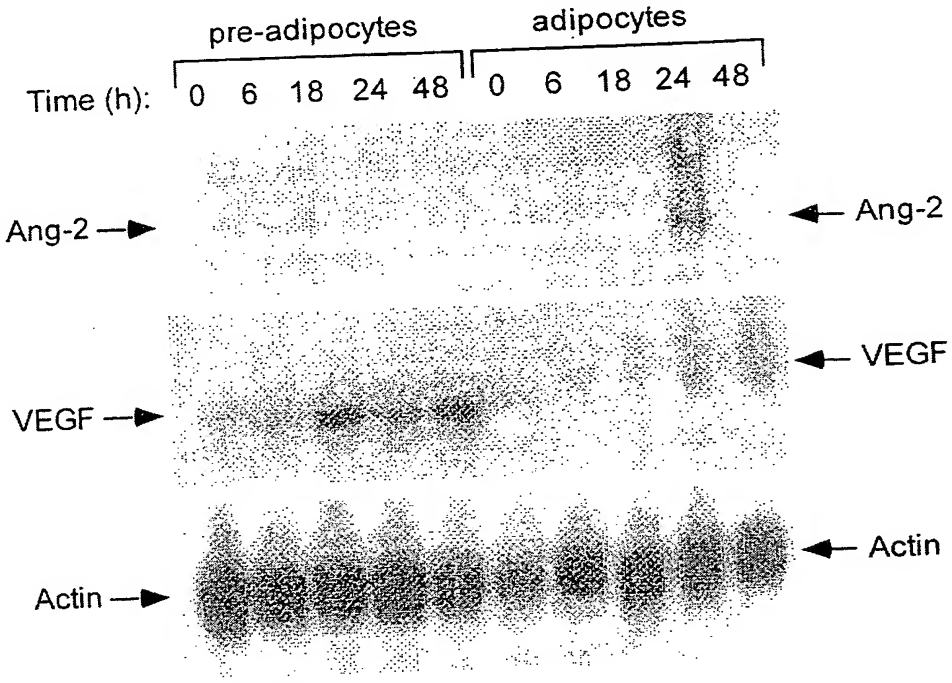
pre-adipocytes
adipocytes
Time (h):
0 6 18 24 48 0 6 18 24 48
Ang-2
Ang-2
VEGF
VEGF
Actin
Actin

FIGURE 7

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(57) Abstract: Disclosed is the use of leptin, optionally together with VEGF inhibitors, in inhibition of endothelial cell proliferation and modulation of angiogenesis.

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(A61K38/22,31:185),(A61K38/22,31:195),(A61K38/22,38:19),

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, WPI Data, PAJ, MEDLINE, CANCERLIT, EMBASE, CHEM ABS Data, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 27286 A (PROGENITOR INC) 31 July 1997 (1997-07-31) page 29, line 14 - line 30 page 31, line 1 -page 34, line 8 page 52, line 11 -page 54, line 2 claims 26-31 ---	1-10
A	SIERRA-HONIGMANN M ROCIO ET AL: "Biological action of leptin as an angiogenic factor." SCIENCE (WASHINGTON D C), vol. 281, no. 5383, 11 September 1998 (1998-09-11), pages 1683-1686, XP002161601 ISSN: 0036-8075 the whole document --- -/--	1-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 February 2001	13/03/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Stein, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/IL 00/00525

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 (A61K38/22,38:48)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LAFONTAN MAX ET AL: "Leptin and angiogenesis." M-S (MEDECINE SCIENCES), vol. 15, no. 3, March 1999 (1999-03), pages 382-386, XP000982571 ISSN: 0767-0974 the whole document ---	1-10
A	WO 98 48831 A (RUBINSTEIN MENACHEM ;COHEN BATYA (IL); BARKAN DALIT (IL); YEDA RES) 5 November 1998 (1998-11-05) page 2, line 20 -page 9, line 3 claims 1-27; examples 5-9 ---	1-10

-/--

[X] Further documents are listed in the continuation of box C.

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° Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

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Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Stein, A

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INTERNATIONAL SEARCH REPORT

Int :ional Application No
PCT/IL 00/00525

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 59614 A (UNIV YALE ;SIERRA HONIGMANN ROCIO M (US)) 25 November 1999 (1999-11-25) the whole document, especially examples 2,3,5-13 and claims 1-9	1-10

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-4,6-10 partially

Present claims 1-4 and 6-10 relate to compounds defined by reference to a desirable characteristic or property, namely the inhibition of VEGF action or synthesis or the inhibition of angiogenesis. However these claims lack any structural and essential characteristics of the compounds.
The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compounds by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the compounds mentioned on page 18 lines 3-9, page 18 lines 20-22 and claim 5 of the application.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IL 00/00525

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9727286 A	31-07-1997	AU 1831197 A	20-08-1997
		CA 2244693 A	31-07-1997
		EP 0892849 A	27-01-1999
WO 9848831 A	05-11-1998	AU 7076298 A	24-11-1998
		BG 103832 A	31-10-2000
		CN 1261802 T	02-08-2000
		EP 0981365 A	01-03-2000
		HU 0002427 A	28-12-2000
		NO 995267 A	28-12-1999
		PL 336582 A	03-07-2000
		SK 147199 A	12-06-2000
		ZA 9803608 A	02-11-1998
WO 9959614 A	25-11-1999	AU 4672199 A	06-12-1999

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 March 2001 (15.03.2001)

PCT

(10) International Publication Number
WO 01/18040 A3

(51) International Patent Classification[7]: A61K 38/22, 48/00, A61P 3/00, 9/00, 15/00, 35/00, 43/00 // (A61K 38/22, 31:52) (A61K 38/22, 38:17) (A61K 38/22, 31:185) (A61K 38/22, 31:195) (A61K 38/22, 38:19)

(21) International Application Number: PCT/IL00/00525

(22) International Filing Date: 4 September 2000 (04.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
131739 5 September 1999 (05.09.1999) IL
132312 10 October 1999 (10.10.1999) IL

(71) Applicant *(for all designated States except US)*: YEDA RESEARCH AND DEVELOPMENT CO. LTD. [IL/IL]; Weizmann Institute of Science, P.O. Box 95, 76100 Rehovot (IL).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: RUBINSTEIN, Menachem [IL/IL]; 16 Hatomer Street, 54042 Givat Shmuel (IL). COHEN, Batya [IL/IL]; Rechov Arlosoroff 182, 64923 Tel Aviv (IL). BARKAN, Dalit [IL/IL]; Vilkomitch Street 22/A, 76448 Rehovot (IL).

(74) Agent: EINAV, Henry; Inter-Lab Ltd., Science-based Industrial Park, Kiryat Weizmann, 76100 Ness-Ziona (IL).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

(88) Date of publication of the international search report: 14 June 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/18040 A3

412

(54) Title: USE OF LEPTIN IN INHIBITION OF ENDOTHELIAL CELL PROLIFERATION

(57) Abstract: Disclosed is the use of leptin, optionally together with VEGF inhibitors, in inhibition of endothelial cell proliferation and modulation of angiogenesis.

R. De Luca

COURTESY COPY OF THE

INTERNATIONAL

PRELIMINARY

EXAMINATION REPORT

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
99-79		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL00/00525	04/09/2000	05/09/1999
International Patent Classification (IPC) or national classification and IPC		
C07K14/00		
Applicant		
YEDA RESEARCH AND DEVELOPMENT CO. LTD. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09/03/2001	12.12.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Rojo Romeo, E Telephone No. +49 89 2399 7321

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IL00/00525

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, pages:

1-28 as originally filed

Claims, No.:

1-10 as originally filed

Drawings, sheets:

1/7-7/7 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

☐ copy of the earlier application whose priority has been claimed.

☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-4, 6-10 (partially).

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion

could be formed.

☒ no international search report has been established for the said claims Nos. 1-4, 6-10 (partially).

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	5 (entirely); 4, 6-10 (partially)
	No:	Claims	1-3 (partially)
Inventive step (IS)	Yes:	Claims	
	No:	Claims	5 (entirely); 1-4, 6-10 (partially)
Industrial applicability (IA)	Yes:	Claims	1-8, 10
	No:	Claims	9 (see separate sheet)

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item II

Priority

The right of priority can be acknowledged.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

As mentioned by the ISA, the present claims 1-4 and 6-10 relate to compounds defined by reference to a desirable characteristic or property, namely the inhibition of VEGF action or synthesis or the inhibition of angiogenesis, without giving any structural and essential characteristics of the compounds.

Consequently, the application was searched as far as it concerns the compounds mentioned on page 18 lines 3-9, page 18 lines 20-22 and claim 5.

As a result, the present claims are examined only as far as they concern said compounds.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents cited in the International Search Report:

D1: WO 97 27286 A (PROGENITOR INC) 31 July 1997 (1997-07-31)

D2: SIERRA-HONIGMANN M ROCIO ET AL: 'Biological action of leptin as an angiogenic factor.' SCIENCE (WASHINGTON D C), vol. 281, no. 5383, 11 September 1998 (1998-09-11), pages 1683-1686, XP002161601 ISSN: 0036-8075

D3: LAFONTAN MAX ET AL: 'Leptin and angiogenesis.' M-S (MEDECINE SCIENCES), vol. 15, no. 3, March 1999 (1999-03), pages 382-386, XP000982571 ISSN: 0767-0974

D4: WO 98 48831 A (RUBINSTEIN MENACHEM ;COHEN BATYA (IL); BARKAN DALIT (IL); YEDA RES) 5 November 1998 (1998-11-05)

Document D1 concerns the use of leptin for promoting angiogenesis, alone or in combination with cytokines such as VEGF. Moreover, this document suggests the use of leptin to suppress tumour growth by inducing terminal differentiation of certain tumour cells such as leukemic cells which express the leptin receptor.

D2 discloses the role of leptin as angiogenic factor, discussing the role of leptin-induced angiogenesis may assist in heat dissipation at sites of active thermogenesis in the body, including adipose tissue and may play a role in the modulation of adipose tissue mass.

D3 is a review addressing the role of leptin as angiogenic modulator, stressing its role in the development of the fat tissue but also in the neovascularisation of certain tumours, wound healing and oocyte and embryo maturation. This document also suggests the use of leptin in the control of angiogenesis for the regulation of adipogenesis.

D4 discloses the use of leptin, leptin fusion proteins, leptin muteins, leptin receptor agonists, active fragments or fractions of any one thereof, active analogs or derivatives of any thereof, as an inhibitor of cell proliferation, e.g. as an inhibitor of the proliferation of cancer cells (e.g. human breast carcinoma; see page 3)

The present application concerns the use of leptin in the inhibition of endothelial cell proliferation. The examples show that the injection of leptin in female mice lacking endogenous leptin leads to the regression of blood vessels in the adipose tissue, that leptin induces angiopoietin 2 in adipose tissue and that the use of leptin plus a VEGF inhibitor (CSC) leads to adipose mass reduction.

1. Novelty (Art. 33(2) PCT)
   The use of leptin for the preparation of medicaments was known from prior art. However, not for the inhibition of endothelial cell proliferation. Since leptin was shown in prior art to promote angiogenesis (D1-D3), it is unclear why leptin should have now an opposite effect as that shown in the past. If the leptin used by the Applicant has other technical features than the leptin (sequence, concentrations used/obtained...) and leptin derivatives used in the past and shown to have the opposite effect, then the Applicant should explain what these differences are.

   In addition, the Applicant's attention is drawn to the fact that no data is provided that the inhibition of endothelial cell proliferation is reversible. Thus, claim 1 is drawn as a "result to be achieved" without the indication of the technical features necessary for achieving this result.
   At the time being, novelty cannot be acknowledged for claim 1 and dependent claims 2, 3.

In summary, claims 1-3 are not novel, and thus, not inventive.

2. Inventive step (Art. 33(3) PCT)
The problem underlying the present application is the provision of a composition leading to angiogenesis inhibition. The solution provided by the present application is a composition comprising leptin and a VEGF inhibitor.
VEGF inhibitors were already known in the art as angiogenesis inhibitors. Thus, in the absence of comparative data showing that the combination of VEGF inhibitors with leptin has an improved antiangiogenic activity (especially in the light of the controversial data published), inventive step cannot be acknowledged for claims 4-10.

For the sake of completeness, the Applicant's attention is drawn to the fact that both VEGF inhibitors and leptin have been implicated in tumour inhibition (see D1/D4). Moreover, leptin was already known as an angiogenesis modulator in fat tissue (see D2/D3).

The Applicant's attention is drawn to the fact that the intention of use does not limit the scope of a claim which is directed to a composition. The claim must be interpreted as being directed to a composition per se regardless of its use. Claims 6, 7 and 8 are, as claim 10, directed to a composition comprising leptin and a VEGF inhibitor. No unified criteria exist in the PCT as far as first medical use is concerned. The EPO, for instance, will allow claims in a form such as:"substance or composition X", followed by the indication of use ("for use as a medicament"). Or in the case of known compounds (as it is the case here) drawn as a second medical use.

Consequently, claims 1-10 lack inventive step.

3. Industrial applicability (Art. 33(4) PCT)
For the assessment of the present claim 9 on the question whether it is industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the

manufacture of a medicament for a new medical treatment.

Re Item VI

Certain documents cited

D5: WO99/59614

international publication date:	25.11.99
international filing date:	20.05.99
priority data:	20.05.98

Re Item VII

Certain defects in the international application

Concerning the expression "spirit and scope of the invention" found at page 28, the Applicant's attention is drawn to the Guidelines III-4.3a PCT.

Re Item VIII

Certain observations on the international application

1. Clarity (Art. 6 PCT)

1.1 In the absence of technical features defining these compounds, "leptins homologues or derivatives" have no technical meaning for the skilled person. Concerning this, the Applicant's attention is drawn to the fact that the claims must be clear without the context of the description.

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]/IL 00/00525

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/22 A61K48/00 A61P3/00 A61P9/00 A61P15/00
A61P35/00 A61P43/00 //(A61K38/22,31:52),(A61K38/22,38:17),
(A61K38/22,31:185),(A61K38/22,31:195),(A61K38/22,38:19),

According to International Patent Classification (IPC) or to both national classification and IPC

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A	SIERRA-HONIGMANN M ROCIO ET AL: "Biological action of leptin as an angiogenic factor." SCIENCE (WASHINGTON D C), vol. 281, no. 5383, 11 September 1998 (1998-09-11), pages 1683-1686, XP002161601 ISSN: 0036-8075 the whole document --- -/--	1-10

[X] Further documents are listed in the continuation of box C.

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 February 2001	13/03/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Stein, A

Form PCT/ISA/210 (second sheet) (July 1992)

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 (A61K38/22,38:48)

According to International Patent Classification (IPC) or to both national classification and IPC

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

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"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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Date of the actual completion of the international search	Date of mailing of the international search report
28 February 2001	

Name and mailing address of the ISA	Authorized officer
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International Application No
PCT/IL 00/00525

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 59614 A (UNIV YALE ;SIERRA HONIGMANN ROCIO M (US)) 25 November 1999 (1999-11-25) the whole document, especially examples 2,3,5-13 and claims 1-9	1-10